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CALCIUM-MEDIATED HEMAGGLUTINATION BY SERUM AMYLOID P COMPONENT AND THE INHIBITION BY SPECIFIC GLYCOSAMINOGLYCANS

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Summary: Human serum amyloid P component (SAP) was found to agglutinate erythrocytes in the presence of calcium ion. The hemagglutination was strongly inhibited by hyaluronic acid as well as by heparan sulfate and dermatan sulfate, but not by chondroitin 4-sulfate and keratan sulfate. A specific binding of SAP to hyaluronic acid, heparan sulfate, and dermatan sulfate was also confirmed by the fact that these glycosaminoglycans blocked the binding of SAP to agarose, a specific ligand of SAP.

Serum amyloid P component (SAP) is a normal plasma glycoprotein, which is comprised of 10 noncovalently associated identical subunits, each of mass 23-25 kDa, arranged with face-to-face cyclic pentameric discs (1, 2). Most types of amyloid deposits contain amyloid P component (AP), which is morphologically and immunonogically indistinguishable from SAP (3-11). Reports on amino acid sequence of AP and on the the nucleotide sequence of SAP cDNA have shown that the two molecules were identical (12, 13). Although the pathogenesis of amyloid remains unsolved, the observation that a pyruvate acetal of galactose dissociates AP from isolated amyloid deposits (14) suggests that the binding of AP to unidentified ligands is a cause of its accumulation in the pathological condition. AP is also found in normal human glomerular basement membrane and

ABBREVIATIONS: SAP, serum amyloid P component; AP, amyloid P component; SDS, sodium dodecyl sulfate.

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peripheral, microfibrillar mantle of elastic fibers in the skin and blood vessels of normal adults (15, 16).

SAP/AP has the property of Ca^{2+}-dependent binding to particular ligands including agarose (17), amyloid fibrils (10), fibronectin, C-4 binding protein (18), heparan sulfate, and dermatan sulfate (19). SAP has been found in different vertebrates including plaice, dogfish, marine toad, chicken, mouse, and human (20). The binding property of SAP toward specific ligands and the preservation may imply that SAP/AP has important functions, but the biological role is not yet known.

In this paper we show that SAP has a property of Ca^{2+}-dependent hemagglutinin and that the hemagglutination is inhibited by specific glycosaminoglycans, such as heparan sulfate, dermatan sulfate, and hyaluronic acid. This may imply that SAP is possible to be a connector between cells and specific glycosaminoglycans.

MATERIALS AND METHODS

Purified bovine kidney heparan sulfate, hog skin dermatan sulfate, bovine cornea keratan sulfate, shark cartilage chondroitin 4-sulfate and chondroitin 6-sulfate were purchased from Seikagaku Kogyo, Tokyo, Japan. Human umbilical cord hyaluronic acid was obtained from Fluka and Sepharose 4B was from Pharmacia. Bovine serum albumin was from Sigma. All other reagents were of analytical quality.

SAP was isolated from human sera according to the method of Pepys et al. (17) with a slight modification as described elsewhere (19). The serum lectin specific for penultimate galactose residues was prepared using affinity chromatography as described previously (21). Monospecific antisera to SAP and the lectin were raised by immunization of rabbits with the isolated proteins. Iodination of SAP was performed according to the method of MacFarlane (22).

SDS-polyacrylamide gel electrophoresis of human SAP and the serum lectin were performed according to Laemmli (23) with 10% acrylamide. The samples were dissolved in the sample buffer consisting of 2% SDS, 2.5% 2-mercaptoethanol, 0.001% bromphenol blue, 10% glycerol, and 62.5 mM Tris-HCl, pH 6.80, and treated at 100°C for 3 min. At the end of electrophoresis, protein bands were located by staining with Coomassie Brilliant Blue R-250.

Hemagglutination of rabbit erythrocytes was assayed in microtiter V plates (Cooke Engineering Laboratory Products). A sample of purified SAP (25 ul) was serially 2-fold diluted with 10 mM Tris-HCl, 2 mM $CaCl_2$, 150 mM NaCl, pH 7.5, in a microtiter plate. Then, 25 ul of a 2.5% suspension of freshly prepared rabbit erythrocytes in 10 mM Tris-HCl, 2 mM $CaCl_2$, 150 mM NaCl,

pH 7.5, was added to each well. The microtiter plates were agitated and evaluated for hemagglutination after standing for 2 h at room temperature. The titer of SAP is defined as the final concentration of the end point dilution.

Effects of glycosaminoglycans on the calcium-dependent binding of ^{125}I-SAP to agarose were examined using parallel columns of 0.6 ml Sepharose 4B. Identical amount of ^{125}I-SAP (50 ng, 3534 cpm) with or without 100 ug of glycosaminoglycans was applied on columns of Sepharose 4B equilibrated in 10 mM Tris-HCl, 2 mM $CaCl_2$, 150 mM NaCl, 2% bovine serum albumin, pH 7.5. After columns were washed with 2 ml of the column buffer, bound SAP was eluted with 1.5 ml of Tris-buffered saline containing EDTA (10 mM Tris-HCl, 5 mM EDTA, 150 mM NaCl, pH 7.5) and determined from the radioactivity. 82% of applied ^{125}I-SAP was bound to Sepharose columns under the experimental conditions without glycosaminoglycans. The amount of SAP bound in the presence of glycosaminoglycans was compared with a control without any glycosaminoglycan. Data are expressed as the means±s.e.m. of triplicate determinations.

RESULTS AND DISCUSSION

In the previous paper it has been reported that a human serum lectin which recognized penultimate galactose residues of complex carbohydrates agglutinated erythrocytes from several species of vertebrates (21). Since the amino acid composition of the lectin was almost identical with that of human SAP which was purified by affinity chromatography on agarose and subsequent gel filtration, the identity of the serum lectin to SAP was examined. As shown in Fig. 1 *a* and *b*, the two glycoproteins are indistinguishable by SDS-polyacrylamide gel electrophoresis and by immumoprecipitation tests in agar gel with monospecific antisera to SAP and to the lectin. Next, hemagglutination activity of SAP was tested using microtiter plates. As shown in Fig. 1 *c*, SAP agglutinated rabbit erythrocytes at a concentration of 60 ug/ml in the presence of 2 mM $CaCl_2$. The hemagglutinin titer of SAP was the same as that of the serum lectin described previously (21). No hemagglutination was observed in the absence of calcium (data not shown). Heparan sulfate and dermatan sulfate have recently been found to bind to SAP in a calcium-dependent manner (19). Therefore, it was next examined whether these glycosaminoglycans blocked the hemagglutination by SAP. The results (Table 1) show that heparn sulfate, dermatan

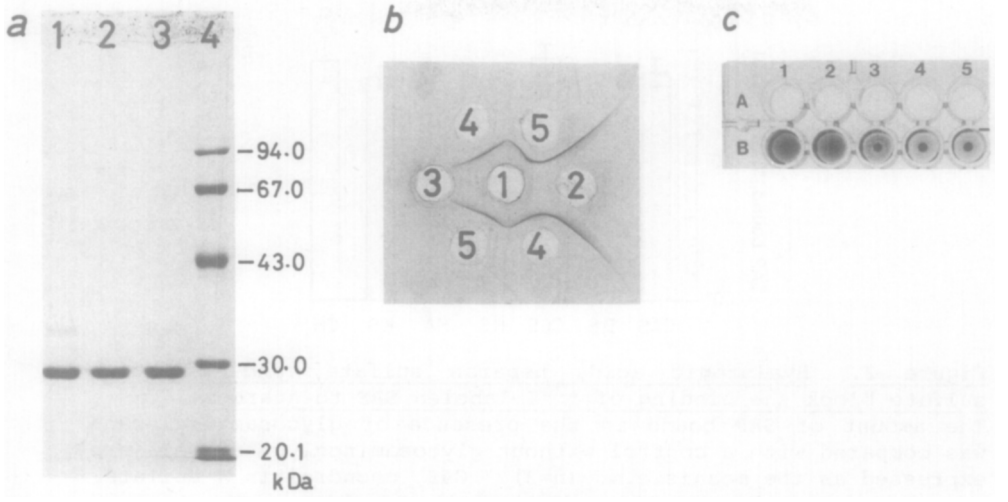

Figure 1. Serum amyloid P component is a hemagglutinin.
a, SDS-polyacrylamide gel electrophoresis of human SAP and the human serum lectin specific for penultimate galactose residues of complex carbohydrates. The samples are: 1, SAP (8 ug); 2, SAP (4 ug) plus lectin (4 ug); 3, lectin (8 ug); 4, standard proteins (Mr in parentheses): phosphorylase b (94000), bovine serum albumin (67000), ovalbumin (43000), carbonic anhydrase (30000), and soybean trypsin inhibitor (20100).
b, Immunodiffusion in agar gel between 1, monospecific rabbit anti-SAP serum; 2, monospecific rabbit anti-lectin serum; 3, rabbit preimmune serum; 4, pure SAP; 5, pure serum lectin.
c, Hemagglutination of rabbit erythrocytes by SAP.

sulfate, and hyaluronic acid potently inhibit the hemagglutination, whereas chondroitin 4-sulfate, chondroitin, and keratan sulfate are less or not inhibitory, suggesting that hyaluronic

Table 1. Heparan sulfate, dermatan sulfate, and hyaluronic acid block hemagglutination by serum amyloid P component

Diluted material	Inhibitory titer
Buffer	0
Chondroitin 4-sulfate	1
Dermatan sulfate	64
Heparan sulfate	64
Hyaluronic acid	256
Keratan sulfate	0
Chondroitin	0

The materials listed were added as 12.5 ul of a solution of 2 mg/ml to 12.5 ul of 10 mM Tris-HCl, 2 mM $CaCl_2$, 150 m M NaCl, pH 7.5, and serially diluted as in MATERIALS AND METHODS. 12.5 ul of SAP solution of 0.24 mg/ml and 25 ul of 2.5% suspension of rabbit erythrocytes were added and the microtiter plates were agitated, incubated for 2 h at room temperature, and then read. The inhibitory titer of a diluted material is defined as the reciprocal of the greatest dilution which blocks hemagglutination.

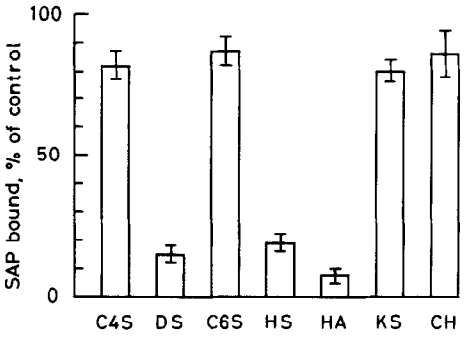

Figure 2. Hyaluronic acid, heparan sulfate, and dermatan sulfate block the binding of ^{125}I-labeled SAP to agarose. The amount of SAP bound in the presence of glycosaminoglycans was compared with a control without glycosaminoglycans. Data are expressed as the means±s.e.m. (n=3). C4S, chondroitin 4-sulfate; DS, dermatan sulfate; C6S, chondroitin 6-sulfate; HS, heparan sulfate; HA, hyaluronic acid; KS, keratan sulfate; CH, chondroitin.

acid has as much affinity for SAP as heparan sulfate and dermatan sulfate. The potent hemagglutination inhibition activity of hyaluronic acid was the unexpected, because it did not affect the binding of heparan sulfate to immobilized SAP (19). The binding of hyaluronic acid to SAP was confirmed by the inhibition test against the binding of ^{125}I-labeled SAP to agarose. As shown in Fig. 2, the calcium-dependent binding of ^{125}I-labeled SAP is strongly blocked by hyaluronic acid as well as by heparan sulfate and dermatan sulfate, suggesting that these glycosaminoglycans have high affinity for SAP, whereas keratan sulfate, chondroitin 4-sulfate, chondroitin 6-sulfate, and chondroitin are much less inhibitory.

Heparan sulfate, dermatan sulfate, and/or hyaluronic acid have been reported to constitute a small but significant and integral part of amyloid tissues (24, 25). It is interesting that only the particular glycosaminoglycans which are found in amyloid-laden tissues show high affinity for SAP, though the binding epitopes on the glycosaminoglycans are yet to be determined. The results presented here favor the view that calcium-mediated association of heparan sulfate, dermatan sulfate, and/or hyaluronic acid with SAP is responsible for the accumulation of the glycoprotein in amyloid-laden tissues. This view is

also supported by the following observations: (1) amyloid deposition takes place concurrently with glycosaminoglycan accumulation in two models of experimental amyloidosis which were induced in mice by daily injections of azocasein or using amyloid-enhancing factor and $AgNO_3$ (26); (2) binding of SAP/AP to amyloid fibril depends on calcium (10); (3) binding affinity of heparan sulfate and dermatan sulfate to SAP increases as calcium concentrations increase (19); and (4) amyloid fibrils in the deposits had associated calcium within their network demonstrated by an electronmicroscopic histochemical technique (27).

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